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An *in vivo* study on anti inflammatory activity of Siddha drug Gandhaga Choornam

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Abstract

To evaluate the acute toxicity and anti inflammatory activities of Ganthaga chooranam (sulphur) in siddha drug. In context, the acute toxicity of Ganthaga chooranam was evaluated by oral administration to make rates as single doses of 5, 50,300, and 2000 mg/kg body weight. General behaviour and toxicity symptoms were observed for 14 days. The anti inflammatory activity was evaluated in carrageenan induced inflammatory paw oedema and activity in male rat. No signs of acute toxicity were observed, including that the LD50 is greater than 2000mg/kg. Ganthaga chooranam (45 and 180mg/kg) significantly reduced oedema formation and at higher dose, the reduction was similar to dexamethasone. The present study shows that Ganthaga chooranam has anti inflammatory properties in rats without causing acute toxicity. These properties observed may be due to the presence of nano substance of sulphur.

Keywords: Acute oral toxicity, Inflammatory, Ganthaga chooranamS

Introduction

Inflammatory diseases are major worldwide problem. non steroidal anti inflammatory drug (NSAIDs) have a number of unfavourable effects and, there is considerable interest is identifying new anti inflammatory agents obtained from siddha medicine used in popular medicine drug. Inflammation is a composite biological reaction of vascular tissue to Harmful stimuli, pathogens, irritants characterised by redness, warmth, swelling and pain. Inflammation is category into either acute or chronic inflammation. Acute inflammation may be an initial response of the body to harmful stimuli. In chronic inflammation the inflammatory response is out of proportion resulting in damage to the body. Cyclo oxygenase(COX) is the key enzymes in the synthesis of prostaglandins, prostacyclins and thromboxanes which are involved in inflammation. Pain and platelet aggregation (Pilottoet al., 2010).

Sulphur has a long history of use for a variety of medical application especially dermatological disorders, as an ingredient in acne ointments and

antidandruff shampoos also as an antidote for acute exposure to radioactive material(linet all.,1988).Sulphur aids in wound healing via keratin and has a history of folk usages a remedy for skin rashes. Topically applied sulphur is keratolytic through the formation of hydrogen sulphide by a reaction that depends on direct interaction between sulphur particles and keratinocytes (Pratselet all., 1992). Topically, sulphur can induce various histological changes including hyper keratosis acanthosis and dilatation of dermal vessels sulphur baths have a long history of used the treatment of psoriasis rheumatic pain and infections, and are still prescribed for asthma by medical doctors in siddha(Gunapadam thathu).due to the use of Ganthaga choornam(sulphur) in folk medicine siddha to combat inflammatory diseases, without scientific evidence of this potential therapeutic application, the aim of the study was to evaluate the acute toxicity and anti-inflammatory effects of Ganthaga choornam(Sulphur) in male rats and to determine the toxicity of this drug after acute exposure.

Materials and Methods

Acute oral toxicity studies where contacted using the OECD(Organization for economic cooperation and development)-Guideline 423 and Institutional animal ethical committees(IAECs) guidelines after 12 hours of fasting, the animals were divided into four groups the treatments were performed by single oral administration at doses 0.500,1000 and 2000 mg/kg body weight of Ganthaga choornam. Animal were observe for science of toxicity during the first 0.5,1,2,4,8 and 12 hours and at every 24 hours for 14 days each. Behaviours parameters, death ,the weight, the amount of water and feed were analyzed. After 14 days of treatment, the animals were weighed and anesthetized (Ketamine and xylsazine,25 and 10 mg/kg, respectively).next, blood samples were collected from the renal vein, with and without anticoagulant(Heparin sodium, cristalia).the blood samples were used to determine the haematology parameters(Total and differential leukocyte count, haematocrit, haemoglobin and erythrocyte count),and the non anti coagulated serum samples were used for biochemical analysis(aspartate amino transferase-AST,alanine amino transferase-AlT, Gamma-glutamyl transferase, creatinine and uria) (Balaninet al., 2011., organisation for economic cooperation and development (OECD),2008),The biochemical parameters were determined using the semi automatic bioplus bio200 equipment(Gold analysis kits).

After that the animals were euthanized and the vital organs(Liver, lung and right kidney) were removed, weighed(absolute and relative to body weight) were determined for the histopathological evaluation of these organs, the samples were fixed in 10% buffered formalin and processed for histological study by light microscopy. The parameters investigated were reversible (degeneration) and irreversible cell damage (necrosis and apoptosis), leucocytes infiltration, congestion, extravasations of blood and fibrosis.

Carrageenan induced rat paw Oedema:

The animals were housed in polypropylene cages with stainless steel top grills having facilities for holding pellet food and drinking water in bottle with stainless steel sipper tube. Each cage contains six rats. All rats had free access to portable water and standard pellet laboratory animal diet ad libitum. paddy husk was used as bedding material. the animals were divided into five groups (six rats/group).localised inflammatory pain was induced in all groups of animals by intraplantar injection of carrageenan(50 micro litre of 3% suspension). Group 1 received vehicle orally. Group 2 received a standard anti analgesic drug, diclofenac sodium(10mg/kg i,p) where as groups 3,4 and 5 received Ganthaga choornam 45 mg/kg ,90 mg/kg, 180 mg/kg b.wb.w.the doses of 45 mg/kg,90 mg/kg and 180 mg/kg b.w were prepared in distilled water, where as diclofenac sodium was dissolved in normal saline.

Different group of rats were orally treated with Ganthaga choornam(45 and 180 mg/kg),or vehicle.another group was treated subcutaneously with dexamethasone (1 mg/kg). After 1 hour the animal received a solution of 50 micro litre carrageenan injection(180 micro gram/paw) in one of the hind paw. The other paw received the same volume of sterile 0.9% saline the thickness

of the paw oedema was measured using a digital micro meter, before the treatment and at 0.5,1,2 and 4 hours after the carageenan injection. results were expressed as micrometer and the difference between basal and post injection values quantified as oedema(Winder et al., 1962) .

Statistical analysis:

Data are presented as mean +- SEM. Difference between groups was evaluated by analysis of variants(one way ANOVA) followed by Newman –keels test. Statically differences were considered to be significant at $p<0.05$.

Results and Discussion

In this 14 days period of acute toxicity evaluation, rates given Ganthaga chooranam leaves in a single dose level of 2000mg/kg body weight, showed no mortality and none of them showed any symptom of toxicity. The behavioural pattern of animal was observed first 5 and 12 hrs and every day for 14 days after the administration and the animals in both vehicle treated and Ganthaga chooranam treated groups were normal and did not display significant changes, skin effect, breathing, defecation, postural abnormalities, impairment in food intake and water consumption and yellowing or loss of hair, compared to negative control group(rats no treated). Neither mortality, nor tremors nor convulsion were noted after 14 days of treatment.

The mean of rat's body weight was measured on a daily basis for 14 consecutive days. No statistically significant defences were shown among group of rats treated compared with negative control group. The last day of treatment, animals were anesthetised and blood collected by cardiac puncture. The rats were sacrificed and liver, heart, kidney lung and sexual organs were collected. There were no significant changes in relative organ weight between both control and treated group. The relative liver weights were $2.63_{+\_0.02\ g/100g\ of}$ b.w and 2.73+_0.01 g/100g of b.w for control and treated group respectively. Values for kidney were 0.35+_0.03g/100g of b.w and 0.37 +_0.02 g/100g of b.w for control and treated group, respectively. The results revealed that the essential organs as liver and kidney were not adversely affected throughout the treatment. Macroscopic analysis of target organs of treated animals did not show significant changes in colour, volume and texture when compared with the control group.

The Ganthaga chooranam was very effective in causing inhibition of the paw volume in the carrageenan induced paw oedema in rat. The anti inflammatory activity at test doses of 37.5,75,150 and 300 mg/kg body weight of Ganthaga chooranam is presented in table 1 with the average volume of carrageenan induced rat paw oedema. The percent protection of inflammation is presented in table 2. The injection of carrageenan in paw created an inflammatory oedema which increased gradually. Gandhaga chooranam significantly inhibitory values of oedema at 3hrs post carrageenam were 125.9%, 113.4% and 111.0% for 45, 90 and 180 mg/kg of Ganthaga chooranam respectively. Diclofenac sodium (10mg/kg) gave a percentage inhibition of 89.11%. gandhaga chooranam showed a significant effect even at the smallest dose (45mg/kg), which inhibited paw oedema by 78.71% at the 2^{nd} hour after carrageenan administration. Vogel and vogel (1997) reported that in vascular permeability assay, mediators of inflammation released following stimulation. Leads to dilatation of arterioles and venues and increased vascular permeability.

Table 1. Effect of Gandhaga chooranam on carrageenan induced paw oedema in rats of volume of paw oedema

group	Mean paw volume before carrageenan injection	Paw volume after induction with carrageenin Increase in paw voliume (ml) after carrageenan injection (mean+- SEM)			(%) percentage inhibition of oedema		
	0 min	30 min	1 hr	2hr	30 min	1hr	2hr
Control	3.30±0.03	6.58±0.1	7.47±0.07	7.6±0.06	99.39%	126.3%	130.3%
standard	3.33±0.05	6.68±0.12	7.455±0.13	7.57±0.13	100.6%	123.7%	127.3%
G.C LD 45mg/kg	3.39±0.07	6.27±0.16	7.20±0.11	7.37±0.07	82.89%	112.3%	117.4%
G.C MD 90mg/kg	3.57±0.22	6.38±0.16	7.29±0.21	7.34±0.14	78.71%	104.2%	105.6%
G.C HD 180 mg/kg	3.63±0.21	6.65±0.29	7.33±0.19	7.44±0.19	84.50%	101.9%	104.9%

Table 2. Effect of Gandhaga chooranam on carrageenam induced paw oedema in rats of percentage of inhibition inflammation

group	Mean paw volume before Carrageenan injection	Paw volume after induction with carrageenin Increase in paw volume (ml) after carrageenan injection (mean+- SEM) percent inhibition of oedema			(%) percentage inhibition of oedema			
	3hrs	4hrs	5hrs	6hrs	3hrs	4hrs	5hrs	6hrs
Control	7.87±0.03	8.08±0.09	8.24±0.03	7.88±0.02	138.4 %	144.8 %	149.6 %	138.7 %
standard	7.79±0.13	8.0±0.15	6.83±0.15	5.63±0.21	133.9 %	141.5 %	105.1 %	69.06 %
G.C LD 45mg/kg	7.66±0.04	7.90±0.04	6.91±0.07	6.20±0.20	125.9 %	109.1 %	103.8 %	73.74
G.C MD 90mg/kg	7.62±0.12	7.83±0.13	6.86±0.21	5.89±0.22	113.4 %	119.3 %	92.15 %	64.98
G.C HD 180 mg/kg	3.63±0.21	6.65±0.29	7.33±0.19	7.44±0.19	111.0 %	115.7 %	87.05 %	60.33 %

Conclusion

Ganthaga chooranam up to the dose level 2000mg/kg body weight did not produceany toxic effects or deaths. The Gandhaga chooranam was well tolerated by the rats. It did not alter body weight, feed and water consumption. The organ weight, biochemical and haematological analysis did not show changes in any of the parameters and examined in animals of both sexes. The acute oral administration of the parameters examined in animals of both sexes. The acute oral administration of the Ganthaga chooranam was safe and not toxic in a single dose.

Finally, the results of present study show that Gandhaga chooranam has anti inflammatory activity. Therefore, the practice of drinking the infusion of the plant for treatment of rheumatism, arthritis and body joints pain by traditional medical practitioner is not totally out of place.

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